

RESEARCH ARTICLE

MicroRNA-200b deficiency is not sufficient to increase susceptibility to allergen-induced airway inflammation and dysfunction in mice

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Abstract

MicroRNA-200b (miR-200b) has emerged as a therapeutic option for reducing inflammation and airway dysfunction in asthma. miR-200b belongs to a family of miRNAs that regulate epithelial-to-mesenchymal (EMT) transition and IL-33 abundance. In asthma, miR-200b abundance is reduced in the airways and is correlated with disease severity. In addition, prophylactic treatment with a miR-200b mimetic reduces airway inflammation and airway dysfunction in a mouse model. However, it is unclear whether miR-200b deficiency is sufficient to drive airway dysfunction and airway inflammation in asthma. Here, we show that male and female mice deficient in miR-200b do not display heightened airway inflammation or alterations in lung function that are characteristic of asthma. Following sensitization with house dust mite (HDM), female miR-200b knockout (KO) mice have elevated total lung resistance and male miR-200b KO have increased airway resistance. However, neither male nor female miR-200b mice display any changes in methacholine sensitivity or responsiveness and do not have enhanced HDM-induced airway inflammation. Collectively, these findings suggest that loss of miR-200b does not drive airway inflammation and airway dysfunction in mice. Thus, although treatment with exogenous miR-200b may ameliorate inflammation in asthma, deficiency of miR-200b is not likely driving pathobiology in asthma.

NEW & NOTEWORTHY MicroRNA-200b regulates the abundance of key asthma-related genes. However, loss of miR-200b does not potentiate allergic asthma in a mouse model, suggesting that miR-200b deficiency may not be sufficient to drive of asthma pathogenesis.

allergic asthma; inflammation; lung; microRNA-200b; sex differences

INTRODUCTION

Asthma is a chronic respiratory disease, characterized by bouts of recurrent wheeze and breathlessness caused by airway narrowing. Hallmark features of asthma include airway inflammation, airway hyperresponsiveness, and airway remodeling, all contributing significantly to disease pathogenesis and progression (1). MicroRNAs (miRNAs) have emerged as important regulators of gene expression in asthma and are attractive targets for novel therapeutics (2). miRNAs act as dimmer switches, able to silence mRNA and alter gene abundance to finetune cellular responses to external stimuli (3). In asthma, miRNAs are important regulators of inflammation and airway remodeling (4, 5). In addition, miRNA profiles in sputum have been associated with asthma severity and type of inflammation (4, 6).

miR-200b belongs to a family of miRNAs that regulate cancer metastasis, epithelial-to-mesenchymal (EMT) transitions, and distal airway development in the lungs (7–9).

miR-200b is enriched in epithelial cells where it tightly controls the abundance of E-cadherin and therefore EMT in tissues (10). Indeed, inhibition of miR-200b results in a loss of E-cadherin and induction of EMT in a colon cancer cell line (11). In asthma, the airway epithelial layer is deficient in E-cadherin, contributing to epithelial permeability and possibly EMT (12). miR-200b also regulates interleukin (IL)-33 abundance, a cytokine that has a genetic association with asthma susceptibility (13, 14). IL-33 is produced in abundance by the airway epithelial cells and drives airway eosinophilia and TH₂ inflammation in asthma (15, 16). Targeting of IL-33 with monoclonal antibodies (itepekimab) can improve lung function and prevent loss of asthma control in people with severe disease (17). miR-200b, through its regulation of EMT and IL-33, is therefore a regulator of asthma pathogenesis.

In people with asthma, miR-200b abundance is significantly decreased in bronchoalveolar lavage (BAL), which correlates with disease severity (13, 18). Mice sensitized to

allergens also display a reduction in miR-200b abundance that is similar to that seen in people with asthma (13). In airway epithelial cells, inhibition of endogenous miR-200b increases IL-33 and TH₂ cytokine production, whereas addition of exogenous miR-200b reduces IL-33 production (13, 19). In mice, prophylactic treatment with exogenous miR-200b inhibits the allergen-induced production of IL-33 and prevents airway inflammation and airway dysfunction (13). Moreover, transgenic mice lacking miR-200b have stiffer lungs and an increase in small airway resistance (7). Although miR-200b is an attractive therapeutic target in the treatment of asthma, it is presently unclear whether this loss of miR-200b is sufficient to drive asthma pathobiology. We hypothesize that mice lacking miR-200b would have greater levels of airway inflammation and airway dysfunction following allergen exposure than wild-type mice. Using a previously established mouse model deficient in miR-200b, we show that neither male nor female mice are more susceptible to house dust mite (HDM) induced airway inflammation or airway dysfunction.

METHODS

Animals

Protocols were approved by the University of Manitoba Animal Care Ethics Board (No. 20-028). Seven- to eight-week-old male and female C57BL/6 miR-200b^{-/-} (KO) mice, generated as previously described, were used (7). Genotyping was done on ear punches via multiplex polymerase chain reaction targeting the miR-200b allele using primers specific for the wild-type (WT) allele (494 bp) and knockout allele (390 bp) as previously described (7). Sample genotyping results are shown in the supplementary materials (Supplemental Fig. S1). These mice were previously confirmed to be deficient in miR-200b and display enhanced protein expression of TWIST and Vimentin, miR-200b targets (7).

HDM Challenge

Mice were anesthetized under isoflurane (4%) and challenged with house dust mite (HDM) *Dermatophagoides pteronyssinus* (Greer Labs) at 25 µg of protein in 35 µL of sterile saline intranasally (i.n.) five times a week for 2 wk, as previously reported (20). HDM stocks contained 3,278 units of endotoxin per mg of protein.

Lung Function

Forty-eight hours after the final HDM challenge, mice were anesthetized with pentobarbital, and a catheter was inserted into the trachea to measure lung function using the FlexiVent small animal ventilator (Scireq, Montreal, Canada). After assessment of baseline measures of lung function and inspiratory capacity, mice were subjected to a cumulative methacholine challenge to assess changes in methacholine sensitivity and airway reactivity using doubling doses of methacholine from 3 mg/mL to 50 mg/mL.

Sample Collection

Lung lavage (BAL) was collected by filling the lungs with two 1 mL aliquots of sterile saline. The cell pellet was collected by spinning the samples at 100 g for 10 min. BAL was

aliquoted and stored at -20°C for future analysis. The cell pellet was resuspended in 1 mL sterile saline for quantification of total and differential cell influx into the airways.

Total and Differential Cell Counts

Total cells per milliliter of lavage were estimated using a hemocytometer. Differential cell counts were performed on slides stained with modified Wright-Giemsa stain (HEMA3 Stat Pack, Fisher Scientific). Cell distributions were analyzed by identifying and counting eosinophils, neutrophils, lymphocytes, and macrophages as previously described (20, 21).

Lavage Supernatant Analysis

The abundance of cytokines and chemokines in the BAL was quantified using a multiplexed array (Eve Technologies, Calgary, AB, Canada). Specifically, a 32-plex cytokine array and a 12-plex TH₁₇ array. Analytes were included for analysis if there was detectable protein (above the lower limit of detection) in at least 75% of the lavage samples from one group. Analytes not meeting this criterion included IL-12p40 and IL-13.

Statistical Analysis

Maximal change in lung function following each dose of methacholine was used to generate a methacholine dose-response curve for each parameter. To calculate a dose-response slope, an index of methacholine sensitivity, lung function data were normalized to saline baseline values, plotted versus log-dose of methacholine, and fitted with a linear trend line. All data were disaggregated by sex to explore sex-specific differences. Data were analyzed using a two-way ANOVA with factors for genotype (WT/KO), allergen exposure (naïve/HDM), and interaction. Post hoc comparisons were made between genotypes in each allergen group. For methacholine response curves, a two-way ANOVA with post hoc comparisons between WT and KO mice was made for naïve and HDM animals individually. Two-way ANOVA post hoc comparison *P* values were adjusted for multiple comparisons using the Sidak method. Multiple *t* test *P* value adjustment for cytokine volcano plots was done using Benjamini-Hochberg correction. Data are presented as means ± SE, and a *P* value cutoff of 0.05 was used to determine statistical significance. Analyses were performed using Prism GraphPad (version 9). Supplemental data available via Figshare (<https://doi.org/10.6084/m9.figshare.21760526>).

RESULTS

Airway Inflammation and Immune Cell Infiltration

There were no differences in total or differential cell counts in naïve WT or miR-200b KO mice (Fig. 1). Upon acute exposure to HDM, total cells in the lavage increased, regardless of sex or genotype (Fig. 1, A and E). HDM exposure also significantly increased the proportion of eosinophils and lymphocytes within the lavage in both male and female WT or KO mice (Fig. 1, B and C, F and G). Female KO mice had a significant reduction in the fraction of lymphocytes recruited to the lung following HDM challenge (Fig. 1C).

Airway lavage in KO female naïve mice had a significantly higher abundance of IL-21 compared with WT naïve mice

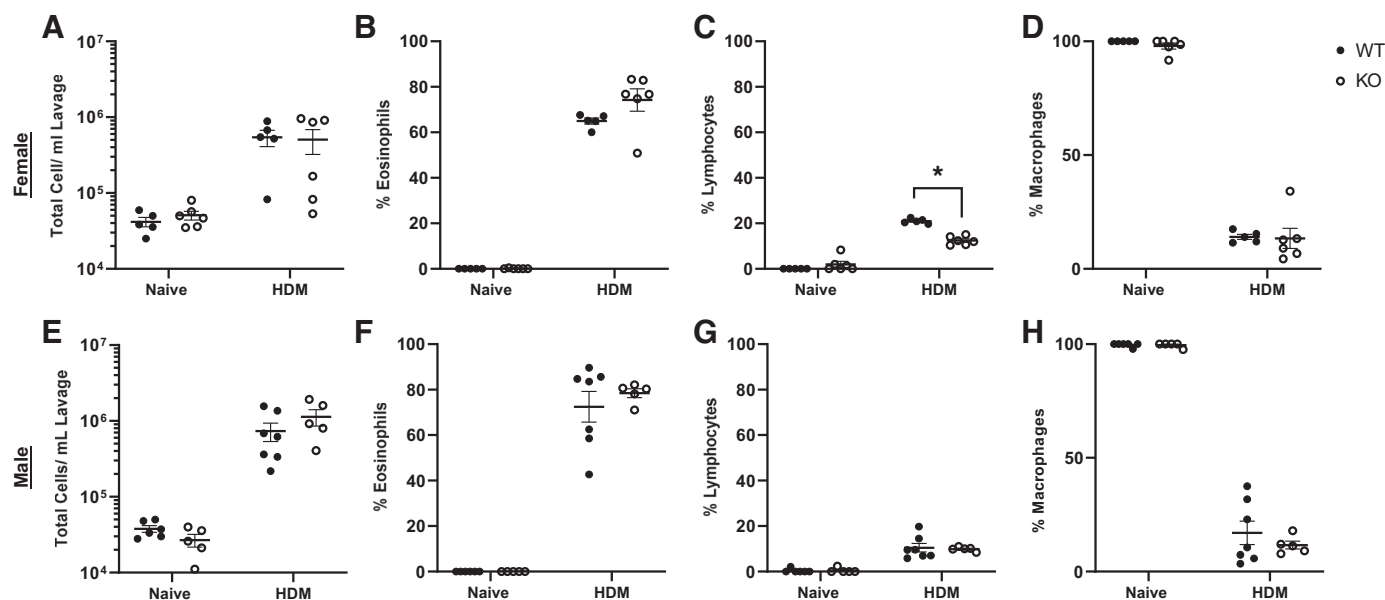


Figure 1. Total and differential lavage cell counts following HDM sensitization. Female total cells/mL of lavage (A), % eosinophils (B), % lymphocytes (C), % macrophages (D) in WT ($n = 5$ naïve and 5 HDM) and miR-200b KO ($n = 6$ naïve and 6 HDM) mice. Male total cells/mL of lavage (E), % eosinophils (F), % lymphocytes (G), % macrophages (H) in WT ($n = 6$ naïve and 7 HDM) and miR-200b KO ($n = 5$ naïve and 5 HDM) mice. * $P < 0.05$ by two-way ANOVA with Sidak's multiple comparisons test. HDM, house dust mite; KO, knockout; WT, wild type.

($P = 0.01$, $\text{padj} = 0.45$, Fig. 2A). Male KO naïve mice had significantly higher levels of monokine induced by gamma interferon (MIG/CXCL9; $P = 0.01$, $\text{padj} = 0.33$) and tumor necrosis factor beta (TNF β ; $P = 0.04$, $\text{padj} = 0.83$) compared with WT naïve males (Fig. 2B). They also had a slight reduction in IL-1 β ($P = 0.05$, $\text{padj} = 0.85$). Importantly, none of these differences were significant after correcting for multiple comparisons. Although HDM challenge increased the abundance of cytokines and chemokines in the lavage, there was very little difference in the cytokine profiles between KO and WT animals. Specifically, only IL-6 was different between male KO and WT HDM animals, with a significant increase ($P = 0.05$, $\text{padj} = 0.87$, Fig. 2D). Again, this difference did not pass multiple comparison correction.

Among cytokines relevant to asthma pathophysiology (Fig. 3), HDM challenge significantly increased the abundance of IL-4, IL-5, IL-6, IL-17, KC/Gro, and TNF α regardless of sex or genotype (Fig. 3). IL-33 abundance was increased by HDM exposure only in female mice, regardless of genotype (Fig. 3). As before, only IL-6 abundance was different between male WT and KO mice (4.85 ± 2.56 vs. 27.28 ± 15.46 pg/mL, Fig. 3). Together, these results suggest that loss of miR-200b largely does not alter the inflammatory profile of HDM challenged airways in mice and is not sufficient to drive changes in airway inflammation on its own.

Baseline Lung Function

There were no differences in lung function in female KO naïve animals, regardless of sex, when compared with naïve WT mice. In female mice, loss of miR-200b increased total lung resistance following HDM challenge (0.85 ± 0.031 vs. 1.39 ± 0.28 cmH $_2$ O-s/mL, Fig. 4A). In male mice, miR-200b KO animals had increased Newtonian resistance, regardless of allergen exposure (Fig. 4F). This difference was not significant following post hoc comparison between naïve animals

(0.20 ± 0.01 vs. 0.31 ± 0.06 cmH $_2$ O-s/mL) or HDM animals (0.24 ± 0.02 vs. 0.36 ± 0.06 cmH $_2$ O-s/mL). Collectively, these results suggest that miR-200b loss in mice might increase airflow resistance in response to HDM, but is generally not sufficient to drive airway dysfunction on its own.

Methacholine Responsiveness

In WT female mice, HDM sensitization significantly increased total lung resistance (R_{RS} , Fig. 5A) and tissue damping (G, Fig. 5C) reactivity to methacholine relative to WT naïve mice (two-way ANOVA, Table 1). HDM sensitization also expectedly increased methacholine sensitivity (Dose response slope) in WT female mice for R_{RS} and G (Fig. 6, A and C). In KO female mice, HDM sensitization did not significantly alter reactivity to methacholine or methacholine sensitivity relative to KO naïve HDM mice (Table 1). When comparing responses to HDM sensitization between WT and KO female, KO female mice had lower methacholine sensitivity compared with WT female mice for G (Fig. 6C). However, there were no other differences between WT and KO mice in response to HDM, indicating that loss of miR-200b did not augment the effects of HDM sensitization on methacholine sensitivity and may have in fact blunted the response.

In WT male mice, HDM sensitization significantly increased R_{RS} (Fig. 5E), Newtonian resistance (R_N , Fig. 5F), and G (Fig. 5G) reactivity to methacholine but not tissue elasticity (H, Fig. 5H) relative to WT naïve mice (two-way ANOVA Table 1). HDM sensitization also significantly increased methacholine sensitivity in WT male mice relative to naïve mice for R_{RS} and G (Fig. 6, E and G). In KO male mice, HDM sensitization significantly impacted reactivity to methacholine for every lung function parameter: R_{RS} (Fig. 5E), R_N (Fig. 5F), G (Fig. 5G), and H (Fig. 5H). Importantly, there were significant differences in HDM-induced increases in R_{RS} and R_N between WT and KO mice. Specifically, WT male mice had higher methacholine

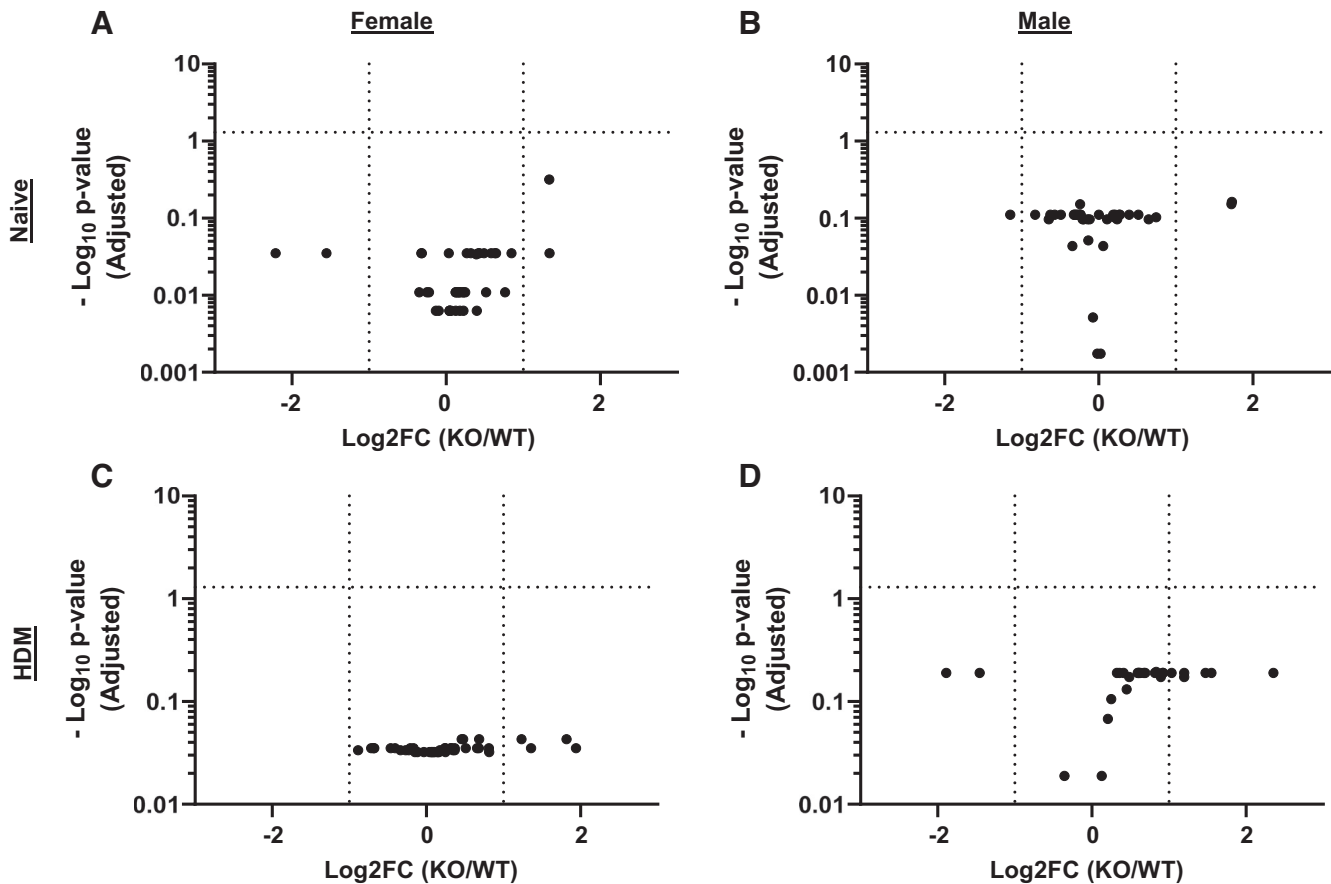


Figure 2. Volcano plots of lavage cytokine and chemokine differences in WT and miR-200b KO mice sensitized to HDM. Comparison of Log₂ fold change (x-axis, KO vs. WT) with adjusted $-\text{Log}_{10}$ *P* values between WT and miR-200b KO female naïve (A), male naïve (B), female HDM (C), male HDM mice (D). *P* values are calculated from multiple *t* tests and adjusted with Benjamini FDR correction. Dashed vertical lines showing $\pm$ twofold change. Dashed horizontal line showing $P < 0.05$. FDR, false discovery rate; HDM, house dust mite; KO, knockout; WT, wild type.

reactivity for R_{RS} following HDM sensitization than KO mice, but KO male mice had significantly higher methacholine reactivity for R_N than WT mice. Although, KO males did not display an increase in methacholine sensitivity for R_N . This suggests that the higher baseline R_N in these mice (Fig. 4F) results in an overall increase in airway narrowing to methacholine but not an increase in sensitivity. In addition, naïve KO male mice had significantly lower reactivity to methacholine for *G* and *H*, signaling the potential for changes to the peripheral airways, independent of HDM sensitization (Fig. 5, *G* and *H*). Finally, WT mice had a significant increase in methacholine sensitivity following HDM for *G* (Fig. 6G) and had significantly greater methacholine sensitivity when measuring R_{RS} compared with KO mice (Fig. 6E). Collectively, these results suggest that loss of miR-200b in male mice marginally increases airway resistance in male mice but does not significantly increase methacholine sensitivity following HDM sensitization.

DISCUSSION

miRNA are important regulators of gene abundance in health and disease, capable of targeting multiple mRNA species. This makes them important drivers of altered mRNA

profiles seen in asthma and attractive therapeutic targets to ameliorate these dysfunctional profiles (2). miRNA-200b abundance is reduced in asthma, and miR-200b and its family member miR-200c are important regulators of IL-33 in human airway epithelial cells and murine lungs (13, 18). IL-33 is an alarmin produced by structural cells in the airway where, upon release, it activates immune cells in the airway including eosinophils, macrophages, and type-2 innate lymphoid cells (ILC2; 15). IL-33 abundance is elevated in bronchial brushings and lavage fluid of lungs from adults with asthma, especially those requiring oral corticosteroids to manage their disease (22, 23). Furthermore, IL-33 abundance in the lavage is inversely related to FEV₁ (23). In mice, neutralizing IL-33 reduces the abundance of eosinophils, ILC2s, and prototypical allergic cytokines (24). Itepekimab, a monoclonal antibody targeting IL-33, was shown in a phase 2 clinical trial to reduce incidences of loss of asthma control and improve lung function (17). We, therefore, hypothesized that loss of miR-200b would result in an increase in IL-33 abundance in the lung, augmented by HDM exposure, resulting in changes in lung function and inflammation indicative of allergic disease. However, we saw no increase in IL-33 abundance in naïve KO mice, regardless of sex, and no augmentation of IL-33 production in response to HDM (Fig. 3). Although a miR-200b mimetic can reduce the production

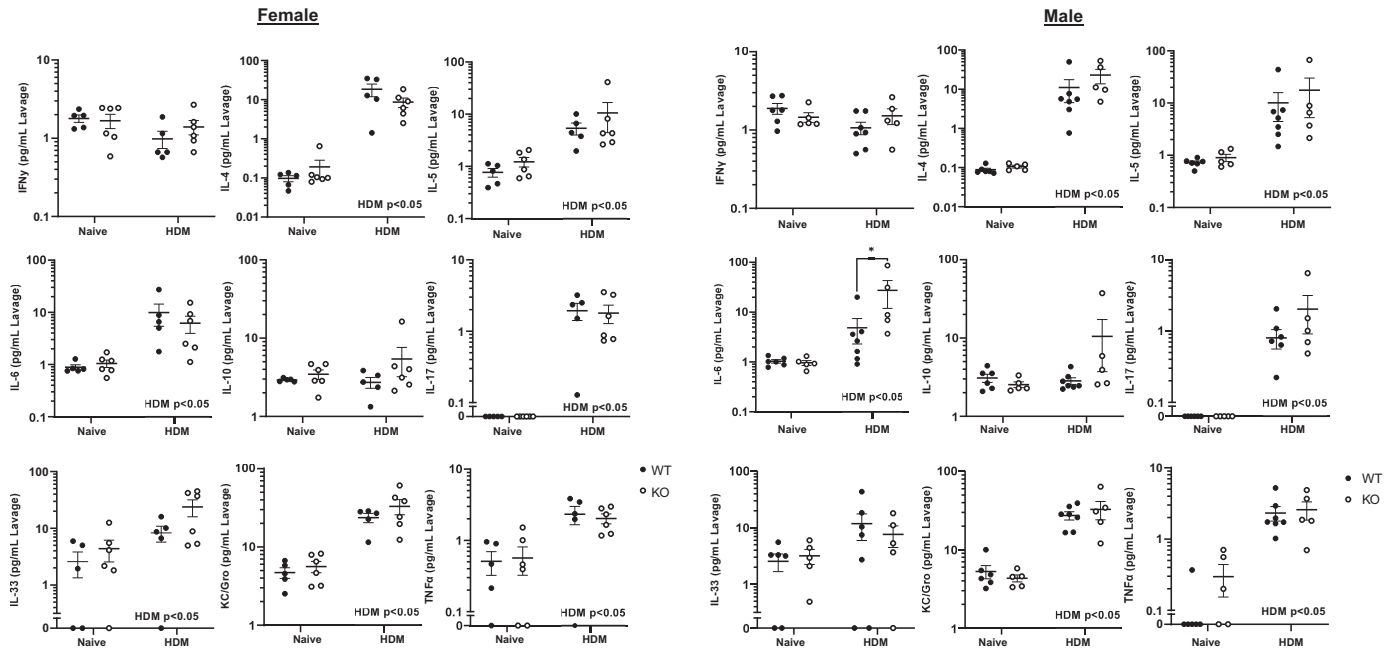


Figure 3. Comparison of key asthma related cytokines and chemokines in WT and miR-200b KO mice. Abundance of IFN γ , IL-4, IL-5, IL-6, IL-10, IL-17, IL-33, KC/Gro, and TNF α in female and male WT and miR-200b KO mice sensitized to HDM. Female mice: $n = 5$ WT naïve, $n = 5$ WT HDM, $n = 6$ KO naïve, and $n = 6$ KO HDM. Male mice: $n = 6$ WT naïve, $n = 7$ WT HDM, $n = 5$ KO naïve, and $n = 5$ KO HDM. $P < 0.05$ by two-way ANOVA with Sidak's multiple comparisons test. Factors from two-way ANOVA that are significant are noted within the figures. HDM, house dust mite; KO, knockout; TNF α , tumor necrosis factor beta; WT, wild type.

of IL-4, IL-5, and IL-13 in an ovalbumin mouse model of allergic asthma, loss of miR-200b did not increase any cytokines normally associated with asthma in naïve mice (Fig. 2; 13). In addition, only IL-6 abundance was augmented in miR-200b KO male mice sensitized to HDM, although only with an unadjusted P value (Fig. 3). Interestingly, we also

saw a reduction in lymphocyte numbers in miR-200b deficient females following HDM challenge. This may suggest that miR-200b is important for lymphocyte recruitment in asthma, however, we have no information on the type of lymphocytes in the airways and so we do not know whether this reduction represents a change in the TH₁/TH₂ balance in

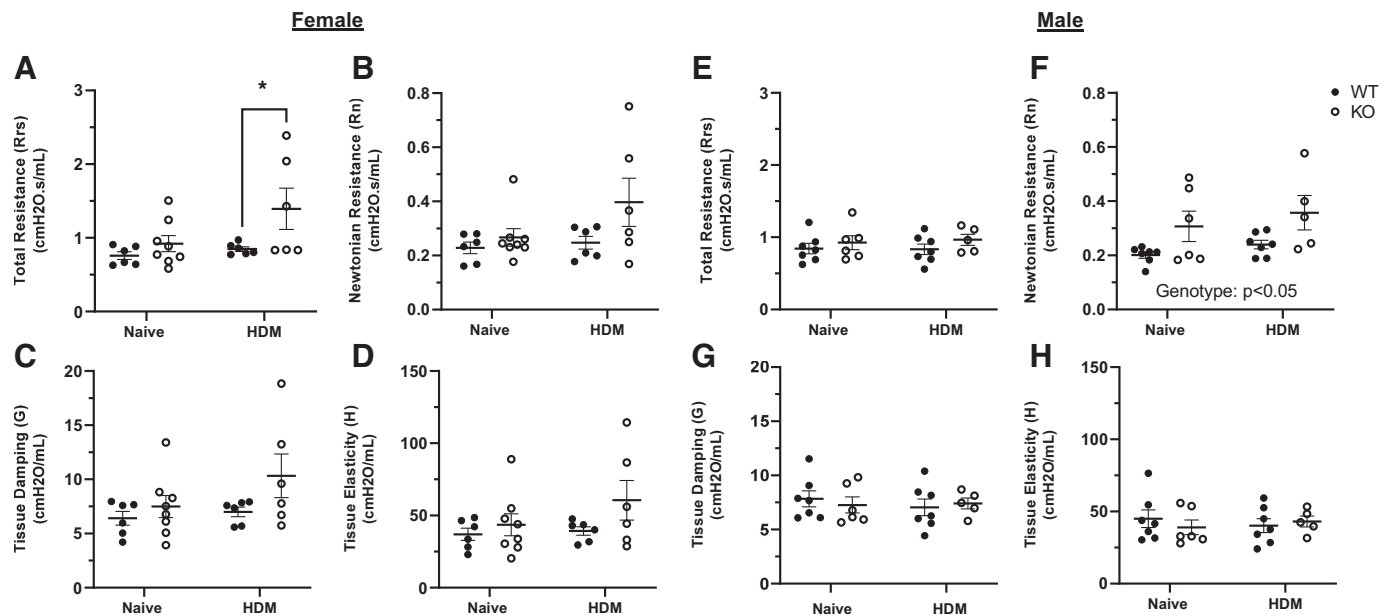


Figure 4. Comparison of baseline line function differences in WT and miR-200b KO mice. Female total resistance (R_{RS}) (A), Newtonian resistance (R_N) (B), tissue damping (G) (C), and tissue elasticity (H) (D). Male total resistance (R_{RS}) (E), Newtonian resistance (R_N) (F), tissue damping (G) (G), tissue elasticity (H) (H). Female mice: $n = 6$ WT naïve, $n = 6$ WT HDM, $n = 8$ KO naïve, and $n = 6$ KO HDM. Male mice: $n = 7$ WT naïve, $n = 7$ WT HDM, $n = 6$ KO naïve, and $n = 5$ KO HDM. $P < 0.05$ by two-way ANOVA with Sidak's multiple comparisons test. HDM, house dust mite; KO, knockout; WT, wild type.

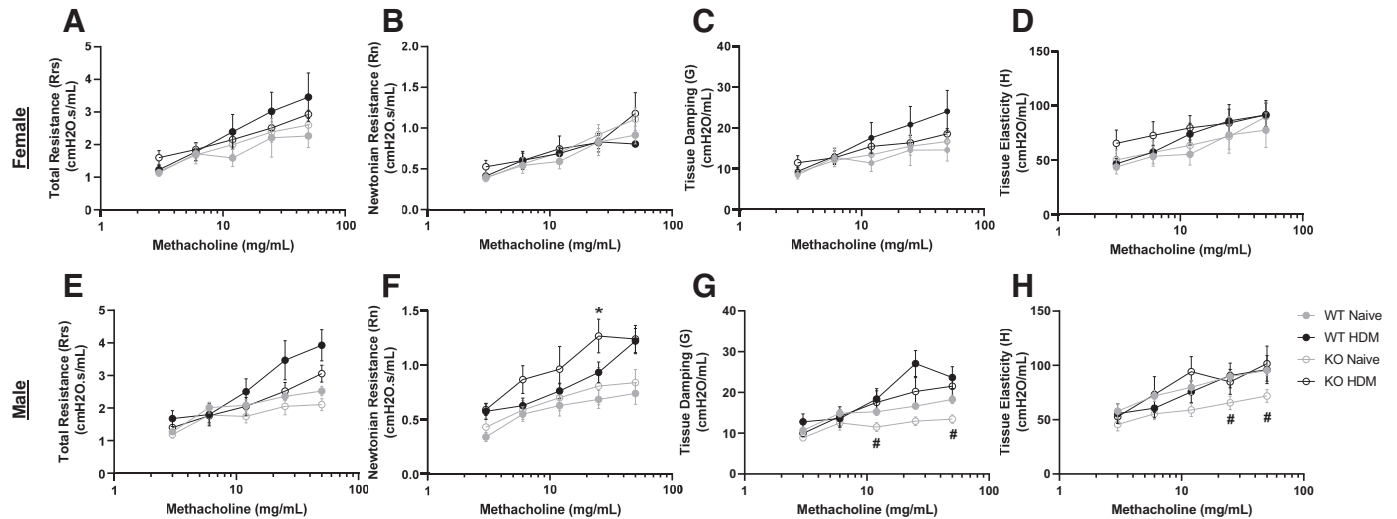


Figure 5. Methacholine response curves in male and female mice sensitized to HDM. Female total resistance (R_{RS}) (A), Newtonian resistance (R_N) (B), tissue damping (G) (C), tissue elasticity (H) (D). Male total resistance (R_{RS}) (E), Newtonian resistance (R_N) (F), tissue damping (G) (G), tissue elasticity (H) (H). Female mice: $n = 6$ WT naïve, $n = 6$ WT HDM, $n = 8$ KO naïve, and $n = 6$ KO HDM. Male mice: $n = 7$ WT naïve, $n = 6$ KO naïve, and $n = 5$ KO HDM. Comparisons made by two-way ANOVA with Sidak's multiple comparisons test; * $P < 0.05$ WT HDM vs. KO HDM, # WT naïve vs. KO naïve.

the airways. miR-200c can also regulate IL-33 abundance and EMT and the miR-200b KO mice used here have normal expression of miR-200c (7, 13, 25). It is therefore possible that miR-200c acts as a compensatory regulator of genes regulated by miR-200b, preventing the hypothesized augmentation of allergic responses in miR-200b deficient mice. Indeed, previously published RNA-sequencing analysis of miR-200b KO lung tissue shows no change in IL-33 transcript abundance further supporting the potential of compensatory mechanisms that limit the impact of miR-200b deficiency on IL-33 induced inflammation (7). More research is required to determine whether miR-200c deficiency alone is sufficient to increase susceptibility to airway inflammation and dysfunction in mice or whether dual-loss of miR-200b and miR-200c is required.

Previous research has indicated that miR-200b deficient male mice have heightened responsiveness to methacholine at the level of the small airways and parenchyma (tissue damping and tissue elastance; 7). In our mice, we could not replicate this finding but did see similar changes in airways resistance (R_N). This increase in R_N was

augmented in male mice sensitized to HDM, resulting in an increase in methacholine responsiveness but not a shift in sensitivity (dose response slope). This suggests that miR-200b KO males have smaller airways than WT males, which can increase airway narrowing in response to methacholine but does not change sensitivity. In female mice, loss of miR-200b did not exacerbate changes in lung function following HDM sensitization, except for baseline total lung resistance (R_{RS}). Importantly, this increase in R_{RS} was variable, with 50% of the female miR-200b KO mice displaying values that were equivalent to the HDM WT mice (Fig. 4A). The lack of broad changes in both baseline lung function and methacholine sensitivity indicates that, in female mice, loss of miR-200b does not influence the established relationship between HDM sensitization and lung dysfunction. Indeed, counter to our hypothesis, loss of miR-200b reduces methacholine sensitivity in male and female mice. This suggests that loss of miR-200b in asthma may be protective against airway hyperresponsiveness. More work is needed to understand how miR-200b abundance in asthma correlates with lung function and methacholine responsiveness.

Sex is an important factor in asthma pathophysiology that is often over-looked in preclinical research. There are important sex-based differences in the response of mice to HDM, highlighting that it is not sufficient to just use one sex in research using animal models (26). A strength of our study is the use of both male and female WT and KO mice, allowing us to explore whether miR-200b deficiency differentially affects HDM-induced airway inflammation and airway dysfunction in mice. We did not do a head-to-head comparison of male and female mouse response to allergen, as this has been done in previous studies (26–28). We did see that HDM-sensitized male miR-200b KO mice had elevated IL-6 abundance compared with WT HDM mice, a difference that was not seen in female mice. However, this difference was only seen with unadjusted P values and no other differences were seen in cytokine abundance

Table 1. Two-way ANOVA results for methacholine response curves in Fig. 5

Parameter	HDM vs. Naive		WT vs. KO	
	WT	KO	Naive	HDM
<i>Female mice</i>				
R_{RS}	A P = 0.04	A P = 0.12	G P = 0.34	G P = 0.49
R_N	A P = 0.82	A P = 0.61	G P = 0.22	G P = 0.17
G	A P = 0.02	A P = 0.18	G P = 0.49	G P = 0.25
H	A P = 0.16	A P = 0.09	G P = 0.43	G P = 0.29
<i>Male mice</i>				
R_{RS}	A P = 0.003	A P = 0.01	G P = 0.04	G P = 0.02
R_N	A P < 0.0001	A P = 0.0004	G P = 0.19	G P = 0.02
G	A P = 0.001	A P = 0.0002	G P < 0.0001	G P = 0.15
H	A P = 0.55	A P = 0.0008	G P < 0.0001	G P = 0.46

A, allergen factor; G, genotype factor; HDM, house dust mite; KO, knockout; WT, wild type. Bold values indicate values with $P < 0.05$.

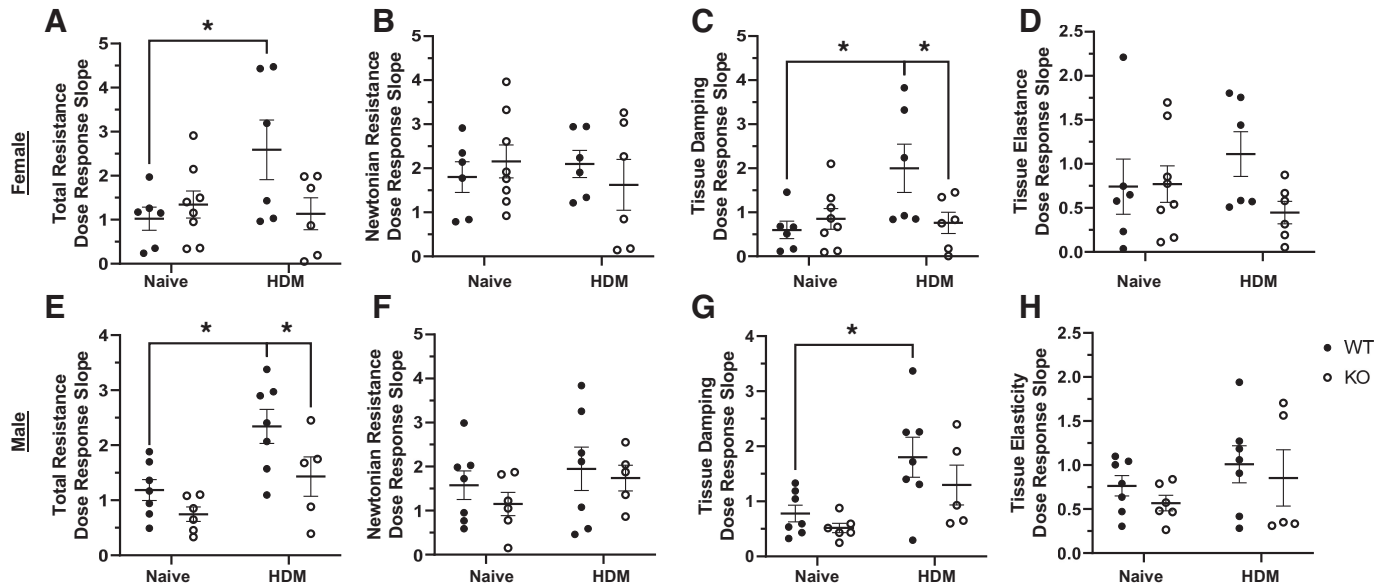


Figure 6. Methacholine responsiveness in male and female WT and miR-200b KO mice sensitized to HDM. Methacholine sensitivity measured using dose response slope of methacholine response curve normalized to baseline values. Female total resistance (R_{RS}) (A), Newtonian resistance (R_N) (B), tissue damping (G) (C), tissue elasticity (H) (D). Male total resistance (R_{RS}) (E), Newtonian resistance (R_N) (F), tissue damping (G) (G), tissue elasticity (H) (H). Female mice: $n = 6$ WT naïve, $n = 6$ WT HDM, $n = 8$ KO naïve, and $n = 6$ KO HDM. Male mice: $n = 7$ WT naïve, $n = 7$ WT HDM, $n = 6$ KO naïve, and $n = 5$ KO HDM. Comparisons made by two-way ANOVA with Sidak's multiple comparisons test; $*P < 0.05$.

between male WT and KO mice. Female naïve miR-200b KO mice displayed heightened IL-21, which may be important in survival of regulatory T cells (29). Following HDM sensitization, KO female mice had higher levels of CXCL9 (monokine induced by gamma interferon/MIG) and tumor necrosis factor (TNF)- β , both of which are markers of TH₁ inflammation (30, 31). Intriguingly, noneosinophilic asthma, which may be driven by a shift toward TH₁ inflammation, is higher in females (32, 33). However, it is important to note that none of these cytokine differences were associated with any noticeable increases in immune cell infiltration into the airways following HDM sensitization or any increases in methacholine responsiveness and airway hyperresponsiveness. Collectively, they suggest that miR-200b deficiency may marginally skew inflammatory profiles but is insufficient to drive allergic airways disease in a mouse model of asthma in either males or females.

We used an acute HDM model that does not cause changes in airway remodeling seen in human asthma (34, 35). Thus, we are unable to determine whether miR-200b is important in influencing airway remodeling in the context of asthma, independent of acute inflammation and airway dysfunction. Interestingly, we saw no neutrophil influx into the airways following HDM challenge. This could be in-part due to the timepoint selected for measurement of airway inflammation and airway function (48-h post HDM), where neutrophils typically peak between 8 and 24 h post HDM sensitization (36). However, it could also be due to a *Nlrp12* mutation in the C57BL/6J strain that this mouse is generated from (37). This mutation causes a defect in neutrophil recruitment to the lung, which may have affected a normal neutrophil response to HDM. The substrain C57BL/6NJ does not harbor this mutation and has normal neutrophil responses in the lung (26, 37). miR-200b is predicted to target 445 genes in mice and 628 genes in humans, with 251 of those overlapping

between the species. These overlap genes are enriched in ontologies for cell migration, tissue morphogenesis, and cell differentiation which is inline with the previously reported role of miR-200b in EMT (7–9). Neither murine nor human miR-200b is predicted to target IL-33, but this may speak to the limitations of microRNA target prediction given that functional evidence exists showing miR-200b activity against the IL-33 untranslated region in mice and humans (13). Finally, our KO model shows reduced expression of miR-200a and miR-429 in miR-200b knockout mice (7). Therefore, we cannot determine what effect the change in abundance of these miRNAs may have had on the response to HDM, but they are likely minimal as overall we saw no major differences between WT and KO mice in response to HDM.

In conclusion, miR-200b is an attractive therapeutic target for asthma due to its ability to regulate IL-33 abundance. However, although there were subtle changes in male airway resistance and cytokine levels, absence of miR-200b did not augment the inflammatory profile and degree of airway dysfunction in HDM-sensitized animals, regardless of sex. This indicates that a decrease in miR-200b abundance in human asthma may not be sufficient to drive asthma pathogenesis. Future research should continue to explore whether treatment with exogenous miR-200b is a therapeutic option for asthma.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL DATA

Supplemental Fig. S1: <https://doi.org/10.6084/m9.figshare.21760526>.

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Graphical abstract image created with BioRender.com and published with permission.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.K. and C.D.P. conceived and designed research; S.K., S.B., Y.L., D.P., and C.D.P. performed experiments; S.K., S.B., Y.L., D.P., and C.D.P. analyzed data; R.K. and C.D.P. interpreted results of experiments; S.K. and C.D.P. prepared figures; C.D.P. drafted manuscript; S.K., R.K., and C.D.P. edited and revised manuscript; S.K., S.B., Y.L., D.P., R.K., and C.D.P. approved final version of manuscript.

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